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Glycosaminoglycan and DNA binding induced intra- and intermolecular exciton coupling of the *bis*-4-aminoquinoline surfen

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Abstract

Despite of the diverse biological activities of the glycosaminoglycan (GAG) antagonist surfen, the molecular details of its interaction with biomacromolecules is poorly understood. Therefore, heparin and DNA binding properties of surfen have been studied by circular dichroism (CD) and UV absorption spectroscopy methods. High-affinity ($K_a \sim 10^7$ M^{-1}) assocation of surfen to the chiral heparin chain gives rise to a characteristic biphasic CD pattern due to the conformational twist of the aminoquinoline moieties around the central urea bridge. At higher drug loading, intermolecular stacking of surfen molecules deeply alters the induced CD profile and also provokes strong UV hypochromism. In contrast to the right-handed heparin template, binding of surfen to the left-helicity chondroitin sulfate chains produces inverted CD pattern. The large UV hypochromism as well as the polyphasic induced ellipticity bands indicate that surfen intercalates between the base pairs of calf-thymus DNA. Extensive CD spectroscopic changes observed at higher drug binding ratios refer to cooperative binding interactions between the intercalated drug molecules. The inherent conformational flexibility of surfen demonstrated here for the first time is important in its binding to distinct macromolecular targets and should be considered for rational drug design of novel GAG antagonists.

Keywords: chondroitin sulfate, circular dichroism; exciton coupling; glycosaminoglycan; helical conformation; heparin, hypochromism; intercalation; nucleic acid; stacking; surfen

Abbreviations: bp, base pair; CD, circular dichroism; CS, chondroitin 6-sulfate; GAG, glycosaminoglycan, ICD, induced circular dichroism

Introduction

Surfen (*bis*-2-methyl-4-amino-quinolyl-6-carbamide, Scheme 1) and its derivatives were originally synthesized in 1937 and patented for their antihelminthic, trypanocidal and antibacterial activities.[1] Surfen was also used as an excipient added to acidic solution of insulin to prepare soluble complexes for therapeutic purposes. Surfen insulins of intermediate and long duration of action had been widely administered in Europe for diabetic patients.[2] Apart from a single study on the development of lymphoma in mice fed with high doses of surfen over a prolonged period,[3] only sporadic cutaneous allergic reactions to surfen-containing depot-insulins have been reported in humans.[4] After many decades, the discovery of the C5a anaphylatoxin inhibitory potential of surfen led to the renaissance of its research.[5] High-throughput screening of 1,990 compounds in the National Cancer Institute Diversity Set and subsequent hit validation identified surfen as the most potent inhibitor of the lethal factor of anthrax toxin.[6] By using the same chemical library, flow cytometric high-content screening indicated that surfen is a promising candidate to enhance the anti-lymphoma activity of a therapeutic monoclonal antibody.[7] In concordance with this result, *in vitro* and *in vivo* inhibitory action of surfen on T cell proliferation has recently been demonstrated.[8]

A unique physicochemical feature of surfen is its avid association to complex, anionic carbohydrates, typically to glycosaminoglycans (GAGs), showing increased binding in parallel with the charge density of GAG chains (chondroitin sulfate < heparan sulfate < dermatan sulfate < heparin).[9] This ability allows surfen to interfere with a number of functional GAG-protein interactions. *In vitro*, surfen significantly attenuated the

signaling effect of fibroblast and vascular endothelial growth factor by blocking their binding to heparan sulfate receptors.[9,10] Similarly, surfen effectively inhibits the heparan sulfate mediated association of endothelial cell membrane proteins. [11] Noticeably, surfen can bind to cationic macromolecules too, such as the so called SEVI (semen-derived enhancer of viral infection) fibrils.[12]

Despite the obvious biological importance, the molecular details of surfen-GAG interactions remain elusive. The double quinoline ring system seems to be essential for this action, since the half-surfen derivative was inactive in fibroblast growth factor binding inhibition assay.[13] Additionally, surfen derivatives obtained by replacement of the $-NH_2$ group with –H, –OH or –OMe substituent do not show heparan sulfate binding that suggests the importance of intermolecular H-bonding in surfen-GAG interactions.[13,14] Except for a fluorescence spectroscopic investigation,[9] no further solution binding studies were performed to assess surfen-GAG interactions. Therefore, employing circular dichroism (CD) and UV absorption spectroscopic methods, this contribution aims to gain insight into the structural features of surfen-heparin association. Since non-covalent complex formation between the cationic surfen molecules and the negatively charged polynucleotide chain can be anticipated, DNA binding of surfen is also evaluated.

Materials and methods

Materials

Surfen (Calbiochem, cat# 362330, purity ≥98.00 %), heparin sodium salt from porcine intestinal mucosa (Sigma, cat# 51551), chondroitin 6-sulfate (CS) sodium salt from shark cartilage (Sigma, cat# C4384) and calf thymus DNA sodium salt (Calbiochem, cat# 2618, 42% G+C content) were used as supplied.

Preparation of surfen and GAG/DNA solutions

Stock solutions of surfen was prepared freshly before each measurement in spectroscopy grade DMSO. The volume of DMSO added into GAG and DNA samples during the CD titrations never exceeded 5% (v/v) and caused negligible effects both on the CD and absorption spectra of the biopolymers. GAG and DNA samples were dissolved in 20 mM Tris-HCl buffer (pH 7.4) containing 50 mM sodium chloride. Due to the polydisperse nature of GAGs, their concentrations were calculated using the molecular weight of the average repeating disaccharide units: 665 and 503 g/mol for heparin and CS, respectively. DNA concentration in term of base pair/L was determined spectrophotometrically by using a molar extinction coefficient of ε_{max} = 13,200 M^{-1} cm^{-1} at 260 nm.[15]

Circular dichroism and UV absorption spectroscopic measurements

CD and UV absorption spectra were recorded on a JASCO J-715 spectropolarimeter at 25 ± 0.2 °C. Temperature control was provided by a Peltier thermostat equipped with magnetic stirring. CD/UV titration experiments were performed in a rectangular quartz cell of 1 cm optical path length (Hellma, USA) either by addition of small aliquots of

surfen stock solution into samples of the macromolecules or *vice versa*, by mixing of small volumes of concentrated GAG/DNA solution to surfen samples. Each spectrum represents the average of three scans obtained by collecting data at a scan speed of 100 nm/min. Absorption spectra were obtained by conversion of the high voltage (HT) values of the photomultiplier tube of the CD equipment into absorbance units. CD and UV curves of surfen-GAG and surfen-DNA mixtures were corrected by the contribution of ligand-free buffer solution of the macromolecules. JASCO CD spectropolarimeters record CD data as ellipticity ('Θ') in units of millidegrees (mdeg).

Calculation of the heparin binding parameters of surfen

The surfen-heparin binding can be quantified by the association constant (K_a):

$$S + HP \rightleftharpoons SHP; \qquad K_a = \frac{[SHP]}{[S][HP]} \quad (1)$$

It is evident that

$$[S] = c_s - [SHP] \quad (2)$$

and

$$[HP] = c_{hp} - [SHP] \quad (3)$$

where c_s and c_{hp} mean the total molar concentrations of surfen and heparin, respectively. Since the formation of surfen-heparin complexes is responsible for the UV hypochromism, it can be written that

$$\varepsilon_0 - \varepsilon = k\,[SHP] \quad (4)$$

Where ε_0 is the molar absorption coefficient (M^{-1} cm^{-1}) of the main UV peak of surfen measured in heparin-free buffer solution, ε is the molar absorption coefficient of the same

peak after addition of increasing amounts of heparin into the sample solution and k is a constant. Using equations 1-4, we obtain

$$(\varepsilon_0 - \varepsilon) = \frac{k}{2}\left(c_s + c_{hp} + K_a^{-1} - \sqrt{\left(c_s + c_{hp} + K_a^{-1}\right)^2 - 4c_s c_{hp}} \right) \quad (5)$$

where K_a is the surfen-heparin apparent association constant.

In order to calculate the value of K_a, non-linear regression analysis was applied using NLREG® (statistical analysis program, version 3.4). Number of surfen molecules bound per a disaccharide unit (n) was estimated by adjusting the molar concentration of surfen (c_s) during the curve fitting procedure. The best curve fit on the UV titration data was achieved by entering 7.4 µM of surfen concentration instead of the experimental value of 26 µM. The ratio of 26/7.4 gives the average number of surfen molecules bound per a disaccharide unit.

Results and discussion

UV absorption spectroscopic features of surfen

Surfen consists of two, methyl substituted 4-aminoquinoline rings connected by a urea core. In pH 7.4 aqueous buffer solution, surfen displays two distinct absorption peaks at 266 and 343 nm and a partially resolved band around 300 nm (Fig. 1). The molar extinction coefficients calculated per aminoquinoline ring are 27,000 (266 nm), 6,500 (300 nm), and 4,600 $M^{-1}cm^{-1}$ (343 nm), respectively (Fig. 1). These values are in good accordance with those reported for 4,6-diaminoquinaldine,[16] the half-surfen molecule, indicating the lack of cross-conjugation between the π systems of the aminoquinoline moieties. Accordingly, surfen can be considered as a bichromophoric molecule composed of two identical, but non-conjugated aromatic units. Linear dichroism measurements showed that in the parent quinoline ring, the electronic transition moment of the most intense UV band (λ_{max} ~ 221 nm) is oriented along the long axis of the molecule.[17] Due to the ring substitutions, this band is red shifted in surfen to ~260 nm (Fig. 1). The UV curves recorded in pH 7.4 Tris-HCl buffer and in 0.05 M HCl solution are highly similar to each other suggesting that surfen molecules are protonated at both pH values (Fig. 1). This finding is supported with the basicity of the ring nitrogen; the pK_a value of 9.17 determined for 4-aminoquinoline[18] indicates the vast majority of the molecules are being positively charged at physiological pH. Deprotonation drastically alters the light absorption of surfen resulting in completely different UV profile (Fig. 1).

Surfen-heparin interaction at low drug/disaccharide binding ratios

CD and UV absorption spectra were recorded at increasing concentrations of surfen in the presence of 1 mM heparin. Addition of the optically inactive ligand induced polyphasic CD curves displaying weaker negative peaks above 275 nm and more intense signals at shorter wavelengths (Fig. 2). The CD couplet below 275 nm consists of a longer-wavelength positive and a shorter-wavelength negative peak with a zero cross-over point at 256 nm, close to the λ_{max} of the associated UV band. Magnitudes of all CD bands enhanced gradually in parallel with the increasing concentration of surfen and showed no significant deviations either in shape or spectral position. Concomitantly with the CD spectral changes, the intense UV peak of surfen is blue shifted by 6 nm in relation to that measured in heparin-free buffer solution (Fig. 2). In contrast, the weak and broad absorption band above 325 nm exhibited 10 nm red shift.

The induced chiroptical signals show that the electronic transitions of surfen are rendered optically active upon interaction with the chiral heparin chain. The characteristic, bisignate pattern of the CD curve at shorter wavelengths is consistent with intramolecular exciton coupling between the π-π^* transition moments of the aminoquinoline moieties. Due to the conformational accomodation of surfen provoked by the heparin binding, these chromophoric units twist around the central urea linker resulting in a chiral conformation of the molecule. According to the exciton chirality rule,[19,20] the observed positive-negative CD spectral motif predicts the prevalence of right-handed, *P*-helical conformation of the heparin bound surfen molecules (Fig. 3).

Of note, exciton interactions affect the absorption spectrum, too.[20,21] 'In-phase' and 'out-of-phase' dipole-dipole coupling of the π-π^* transitions of the aminoquinoline moieties

result in two, a higher and a lower energy transition in the UV spectrum. Due to the slight energy difference between them, these exciton components overlap producing a broadened absorption peak. The blue shift of λ_{max} of surfen (Fig. 2) suggests that in the heparin bound form contribution of the shorter wavelength ('in-phase') exciton component of the main UV band increases. Conversely, 'out-of-phase' interaction of the short axis polarized transition moments is responsible for the red shift of the long-wavelength absorption maximum (Fig. 2).

It should be noted that the stacking of surfen molecules close to each other along the heparin chain in a chiral array could also give rise to a biphasic CD pattern. In analogy with the GAG association of various planar cationic dyes such as methylene blue[22,23] and acridine compounds,[24,25] intermolecular exciton coupling should occur between the aminoquinoline rings of the stacked surfen molecules. In that case, the principal absorption band of the interacting chromophores should exhibit a large intensity reduction (hypochromism) in relation to that of the free form.[25,26] At low drug/disaccharide ratios, however, heparin binding induced only a slight decrease of the ε_{max} of surfen suggesting the intramolecular origin of the induced exciton CD couplet (Suppl Fig. 1, *cf.* Fig. 5).

Surfen-heparin interaction at higher drug/disaccharide binding ratios

Using a much lower heparin concentration, 17 μM instead of 1000 μM, CD/UV titration was performed again. Below the surfen/disaccharide ratio of 1, the CD and absorption curves were very similar to that measured at the high molar excess of heparin (data not shown). Above the equimolar ratio, however, amplitudes of the positive-negative exciton

peaks started to decrease but showed no further significant changes above 25 μM surfen concentration (Fig. 4 and 5). The positive CD band exhibited 4 nm blue shift and its magnitude decreased to one-third. Spectral position of the negative peak around 245 nm remains unaltered, but its intensity also reduced. These CD spectroscopic changes are associated with the bathochromic shift of the main UV band of surfen from 258 nm to 264 nm (Fig. 4). After a first plateau obtained between 17-23 μM ligand concentration, the negative ellipticity values recorded at 277 nm rose again and reached a second saturation phase above 2.6 drug/disaccharide ratio (Fig. 5). Noticeably, increase of the surfen/disaccharide ratio above 1 provoked an abrupt, strong UV hypochromism (Fig. 5, Suppl. Fig. 2). Decrease of the ε_{max} values stopped around 25 μM and remained constant until 35 μM. Above of this ligand concentration (2:1 surfen:disaccharide ratio), a linear increase of the ε_{max} values was seen (Fig. 5).

These data suggest that at binding ratios of ≤ 1, the CD contribution of single surfen molecules accommodated per a disaccharide unit in *P*-helical conformation is dominant. Higher drug loading, however, results in the binding of additional drug molecules which establish π-π stacking interaction with each other (see the next section). Formation of intermolecularly stacked aminoquinoline dimers is accounted for by the large hypochromism measured in the UV spectrum (Fig. 5, Suppl. Fig. 2). Such a drug oligomerization is favourable for intermolecular chiral exciton coupling between the adjacent aminoquinoline rings of the stacked surfen molecules. The intensity increase of the negative CD peak at 277 (Fig. 4 and 5) could be attributed to this mechanism. However, overlap and superposition of the exciton CD signals of intra- and

intermolecular origin makes it difficult to resolve and estimate of their relative contributions.

Estimation of the heparin binding affinity of surfen

The heterogeneous nature as well as the high sensitivity of the induced CD signals to minor stereochemical changes prevent their use for quantitative evaluation of surfen-heparin interaction. In this respect, however, the π-π stacking provoked decrease of the UV absorbance values is much more uniform since it is less dependent on the geometrical features of the binding. Therefore, constant concentration of surfen sample was titrated with increasing amounts of heparin and the UV spectrum was recorded after addition of each aliquot. The absorption data collected by this way were subjected to non-linear regression analysis to estimate the binding parameters (Fig. 6). The value of the association constant ($K_a \sim 10^7$ M^{-1}) indicates the high-affinity heparin binding of surfen molecules. The stoichiometry of the binding shows that about three drug molecules are associated to a repeating disaccharide unit of the polyanionic GAG chain. Remarkably, this value is close to the average negative charge density of the disaccharide unit suggesting 1:1 surfen:sulfate/carboxylate group binding interaction.

Surfen as the helicity sensor of the GAG chains

Both NMR and crystallographic studies showed that the linear polysaccharide chain of heparin adopts a right-handed helical structure.[27] Conformational adjustment dictated by the helicity of the heparin template might be the reason of prevalence of the *P*-helical, right-handed forms of the bound surfen molecules.[28] CD experiments performed with

chondroitin 6-sulfate (CS) lend credence to this assumption. In contrast to heparin, the CS chain acquires a left-handed helical structure.[29] Induced CD curves of surfen measured in the presence of CS are in a mirror image relation to that obtained with the right-handed heparin polymer proving the *M*-helical, left-handed conformation of the bound molecules (Fig. 7). As the molar absorption coefficients show, CS binding does not provoke hypochromism in the absorption spectrum surfen (Suppl. Fig. 3) indicating monomeric binding and thus the intramolecular origin of the measured exciton couplet. Importantly, the large difference obtained for exciton CD intensities at the same drug concentrations suggests that the conformational stereoselectivity of CS for surfen is higher than that of heparin.

Surfen-DNA interaction at low drug/base pair binding ratios

At the high DNA concentration used (214 μM in bp), the CD and absorption spectrum of surfen could only be scanned above 275 nm due to the very strong intrinsic CD and UV activity of DNA at shorter wavelengths. The difference CD curves obtained after subtracting the spectral contribution of the blank DNA solution display a partially resolved negative and two positive ellipticity peaks having similar shape and spectral position to the respective absorption bands (Fig. 8). Amplitudes of the ICD signals increased progressively with the amount of the drug added into the sample solution. Since surfen is achiral itself, these signals can only be generated by its interaction with the chirally disposed nucleobases of the double helix. DNA binding alters the UV bands of surfen as well: a drastic, 70% intensity reduction (hypochromism) and bathochromic shift of the longest-wavelength absorption band can be observed (Fig. 8). In addition, the UV

band centered at 349 nm displays suppressed sub-bands in DNA solution and instead of the shoulder seen in buffer solution, a completely resolved absorption band appears with two peaks at 302 and 310 nm. Of note, DNA binding of the structurally related intercalating drug chloroquine also results in a substantial UV hypochromism.[30,31] Taking into consideration theoretical studies[32] as well as experimental data reported in the literature,[33,34] the UV hypochromism, bathochromic shift, and the ICD band pattern are all indicative to the DNA intercalation of surfen. The ICD signals originate from non-degenerate exciton interactions between the π-π^* electronic transition moments of surfen and the nearest nucleotide bases. Depending on the orientation of the intercalated chromophore, negative ICD signals are predicted for the electronic transitions polarized along the long axis of the intercalation pocket, while converse signs are expected for transitions perpendicular to this direction.[34,35] In analogy with quinoline,[17] the π-π^* transition associated to the high-intensity UV band of surfen is thought to be polarised around the long axis of the molecule. Thus, the observed negative ICD feature allied to this transition (Fig. 8) suggests that the aminoquinoline ring of surfen is inserted parallel to the long axis of the intercalation pocket. However, the binding room between the base pairs (~ 10 Å long) is not large enough to engulf the whole surfen molecule (Scheme 1). Most likely, the major portion of the another aminoquinoline ring protrudes into the DNA groove.

Surfen-DNA interactions at higher drug/base pair binding ratios

Below 0.08 surfen/bp ratio, a polyphasic ICD profile was obtained similar to that measured previously at high molar excess of DNA (Fig. 9). Upon increase further the

drug concentration, however, the long-wavelength positive peaks were gradually converted into negative ones showing distinct shape and spectral position (Fig. 9). In the region of the negative ellipticity band between 267-295 nm, a positive peak emerged followed by an intense negative signal around 250 nm. The zero cross-over point between these opposite signals (263 nm) is close to the blue shifted (262 nm) UV maximum of surfen (Fig. 9). Noticeably, the original UV maximum (274 nm) could also be seen at higher binding ratios as a shoulder on the long-wavelength side of the blue shifted UV band. Additionally, the negative ICD feature associated to the 274 nm UV band is also displayed centered around 283 nm. Importantly, hypochromism of the absorption bands of surfen persists at higher binding ratios too, indicating that the molecules remain intercalated between the base pairs. The overall pattern of the transformed ICD spectrum is reminiscent to that measured at high molar excess of heparin (Fig. 2) which raises the possibility that at higher drug/bp ratios, the intercalated surfen molecules adopt a twisted, *P*-helicity conformation. This structural transition might be initiated through cooperative, allosteric interactions between the drug molecules and may involve the rotation of the aminoquinoline moiety pointing to the DNA groove. The early start of the CD and UV spectroscopic changes at drug/bp ratios $\ll 1$ supports the cooperative binding interaction of surfen molecules. Furthermore, sigmoidal shape of the ε_{max} – [DNA bp] plot obtained from the titration of surfen with concentrated DNA solution also suggests drug-drug binding cooperativity (Suppl. Fig. 4).

Conclusions

CD and absorption spectroscopic data presented in this work demonstrate the binding of surfen to polyanionic biopolymers. Twist of the aminoquinoline moieties around the urea bridge enables surfen molecules to adopt a chiral conformation upon their binding to the helical template of GAG and DNA chains. Association of surfen to heparin and chondroitin sulfate results in characteristic, biphasic ICD patterns of intramolecular origin suggesting the *P*- or *M*-helical disposition of the aminoquinoline rings. Increasing drug loading provokes strong UV hypochromism and substantial transformation of the excitonic CD profile due to stacking of additional surfen molecules to the disaccharide units of heparin. For DNA, the twisted molecular geometry appears only at higher drug loading of the double helix, presumably as the result of cooperative binding interactions of the intercalated surfen molecules. These results imply that the potential pharmacological targets of surfen include not only proteins and GAGs but DNA, too. The inherent conformational adaptability of this drug demonstrated here for the first time is proposed to be important in its biological activities at molecular level.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

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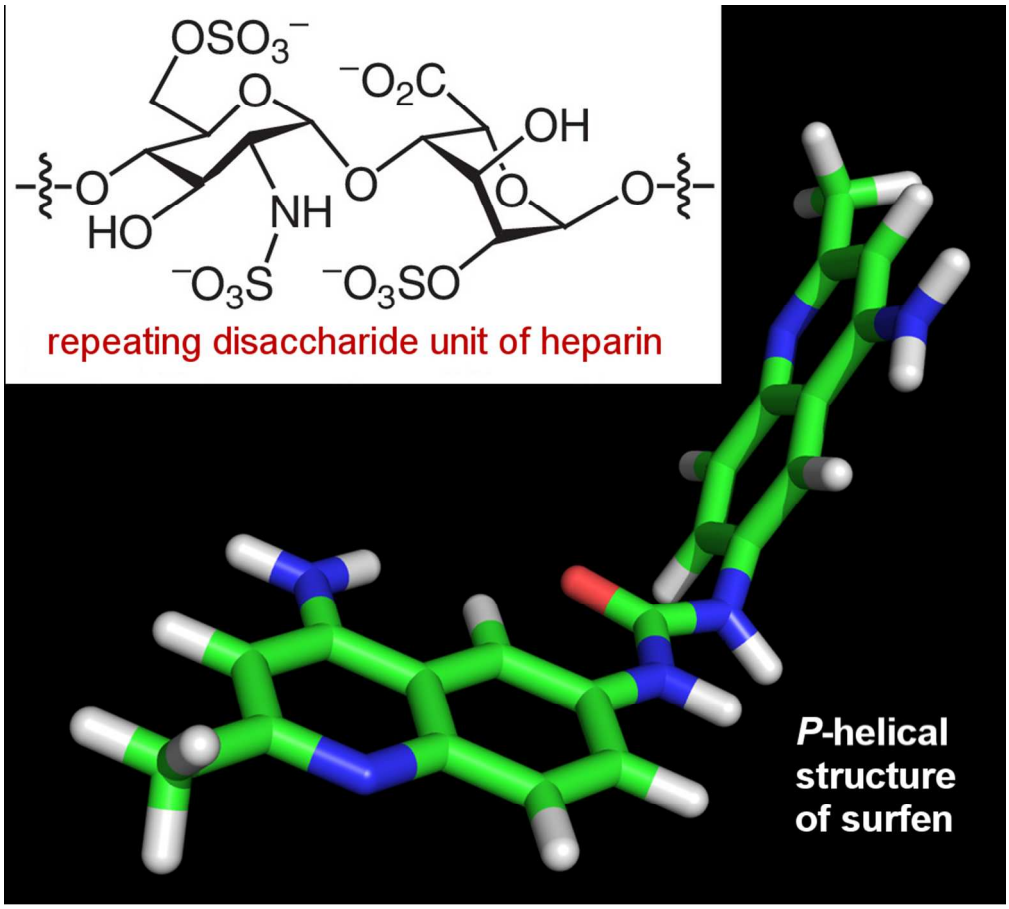

Heparin binding induces intra- and intermolecular exciton chirality of the bichromophoric surfen molecule.
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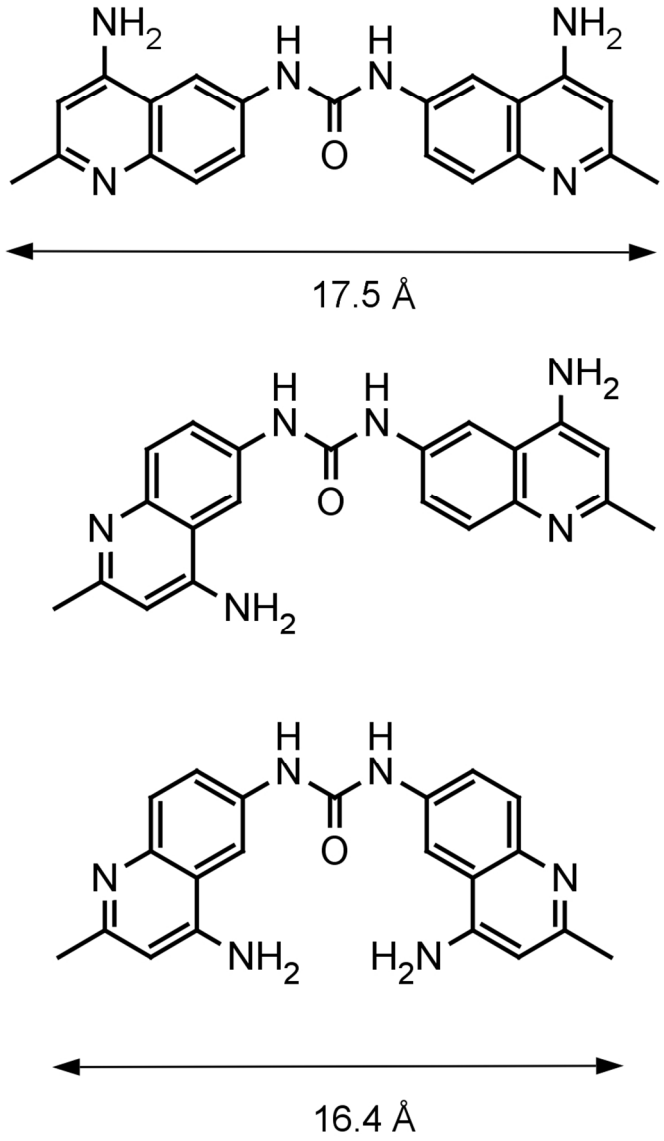

Scheme 1

Symmetrical (top: linear, extended; bottom: crescent-shaped) and non-symmetrical (middle) planar structures of surfen. The crescent-shaped form represents the structure of surfen found in the cocrystal complex of anthrax lethal factor (PDB id. 1PWP).
123x203mm (200 x 200 DPI)

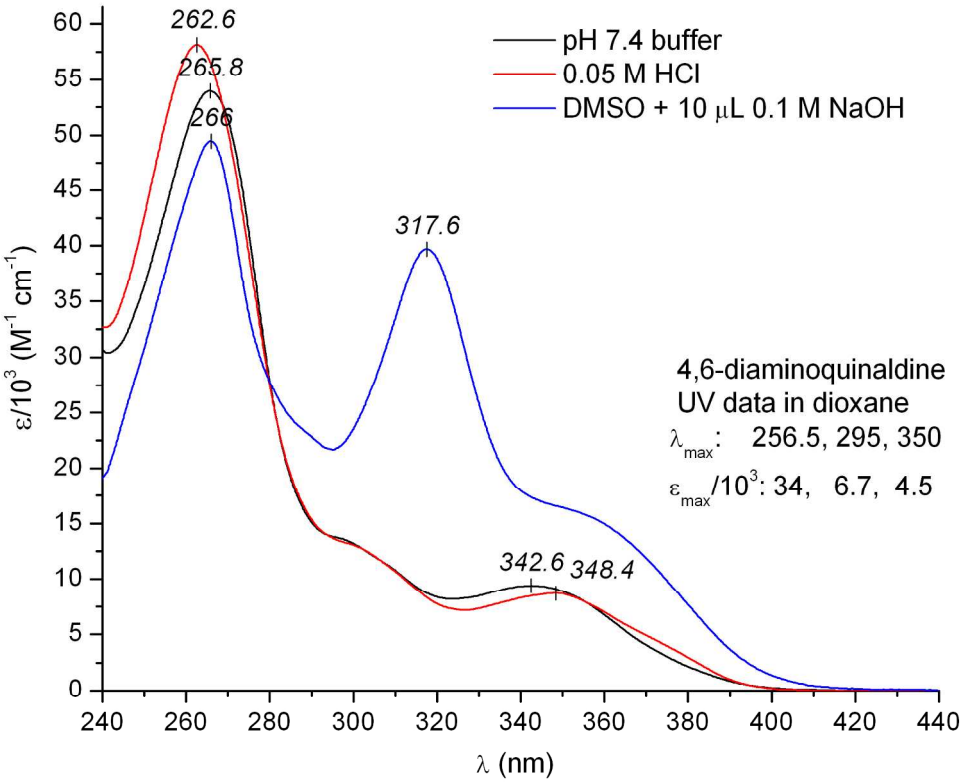

Figure 1
UV absorption spectra of surfen in different solvents (ε_{max} values were calculated by using the molar concentration of surfen). Molar absorption coefficients of 4,6-diaminoquinaldine reported in ref. 16 are shown.
784x648mm (72 x 72 DPI)

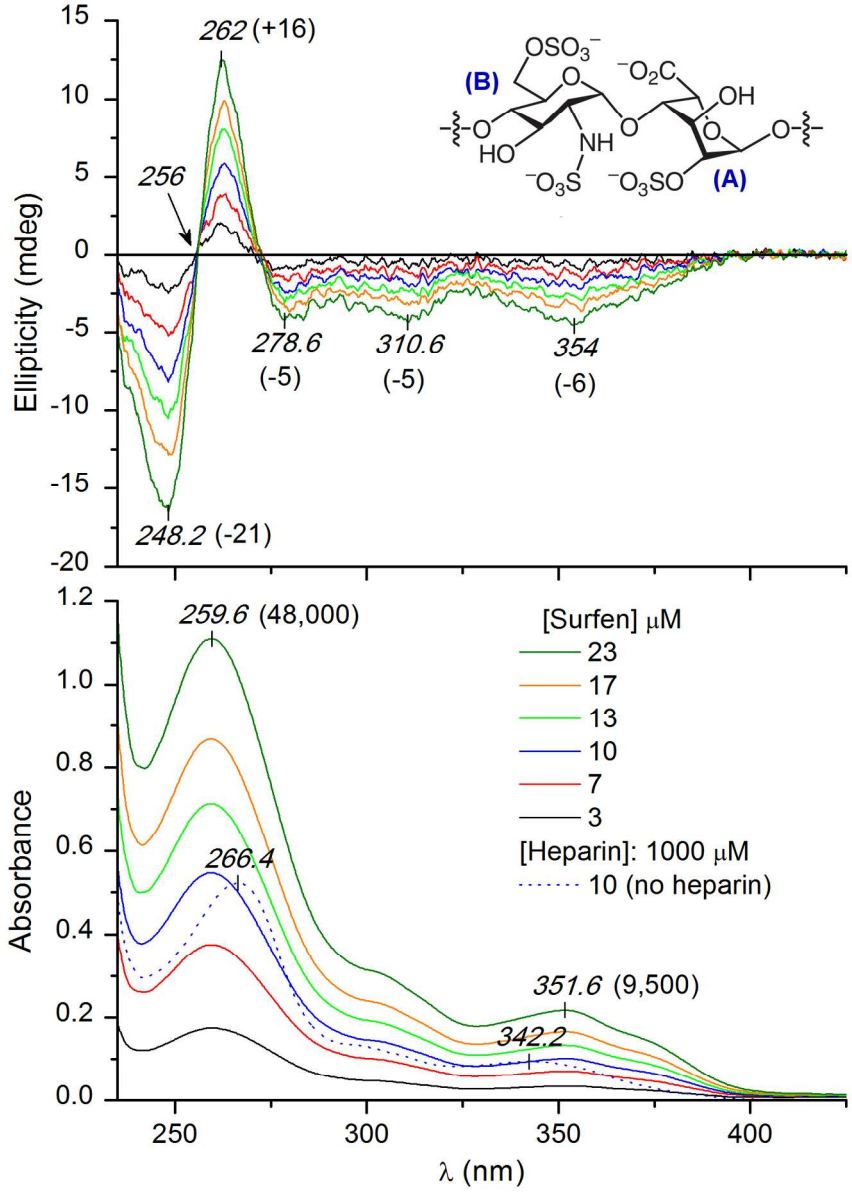

Figure 2

CD and UV absorption spectra of surfen measured at high molar excess of heparin. Dotted line corresponds to the UV curve of surfen recorded in heparin-free buffer solution. Arrow denotes the zero cross-over point. Molar dichroic absorption (±Δε) and molar absorption (ε) coefficients of peak extrema calculated by using actual surfen concentration of the sample are shown in parentheses. Inset displays the repeating disaccharide unit of heparin composed of L-iduronic acid 2-*O*-sulfate (A) and D-*N*-sulfoglucosamine 6-*O*-sulfate (B).

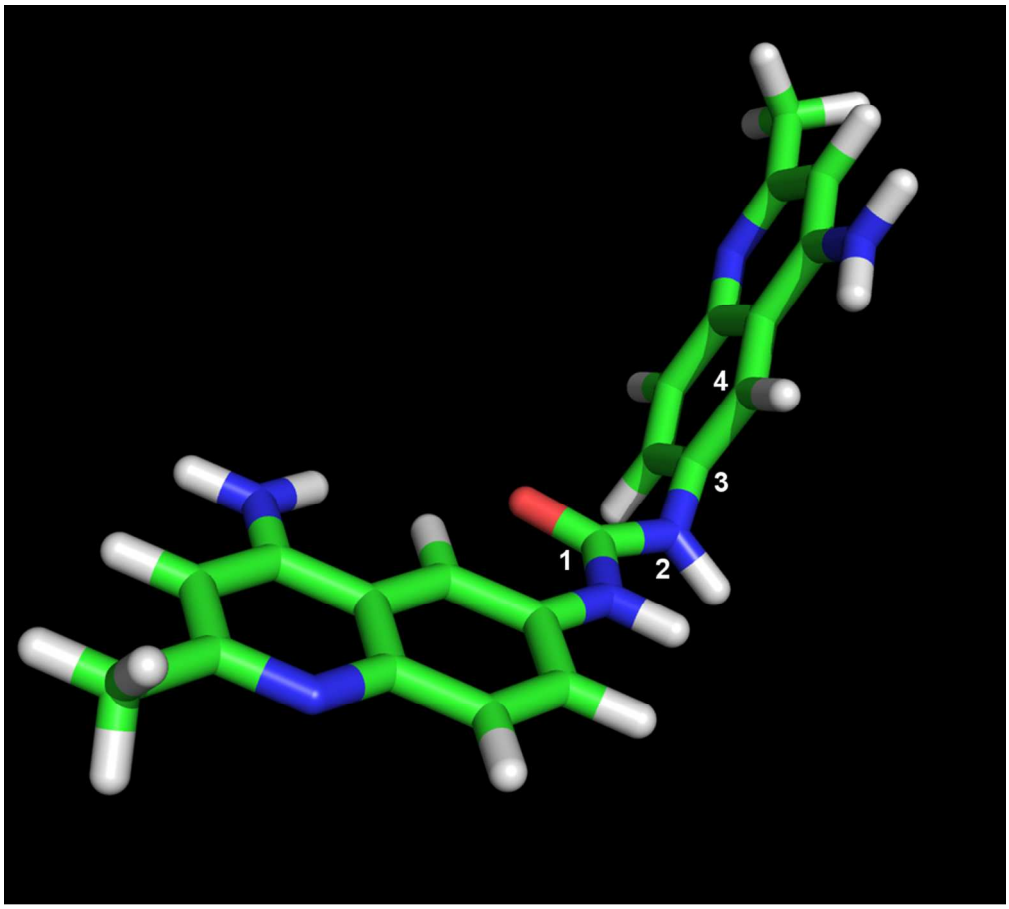

Figure 3
A hypothetical twisted, *P*-helicity conformation of surfen obtained by rotation of the one aminoquinoline ring around the urea core. The dihedral angle of the bonds between the numbered atoms (C1–N2–C3–C4) is +90°. The interplanar angle between the planes of the aminoquinoline moieties is +107°.
211x190mm (127 x 127 DPI)

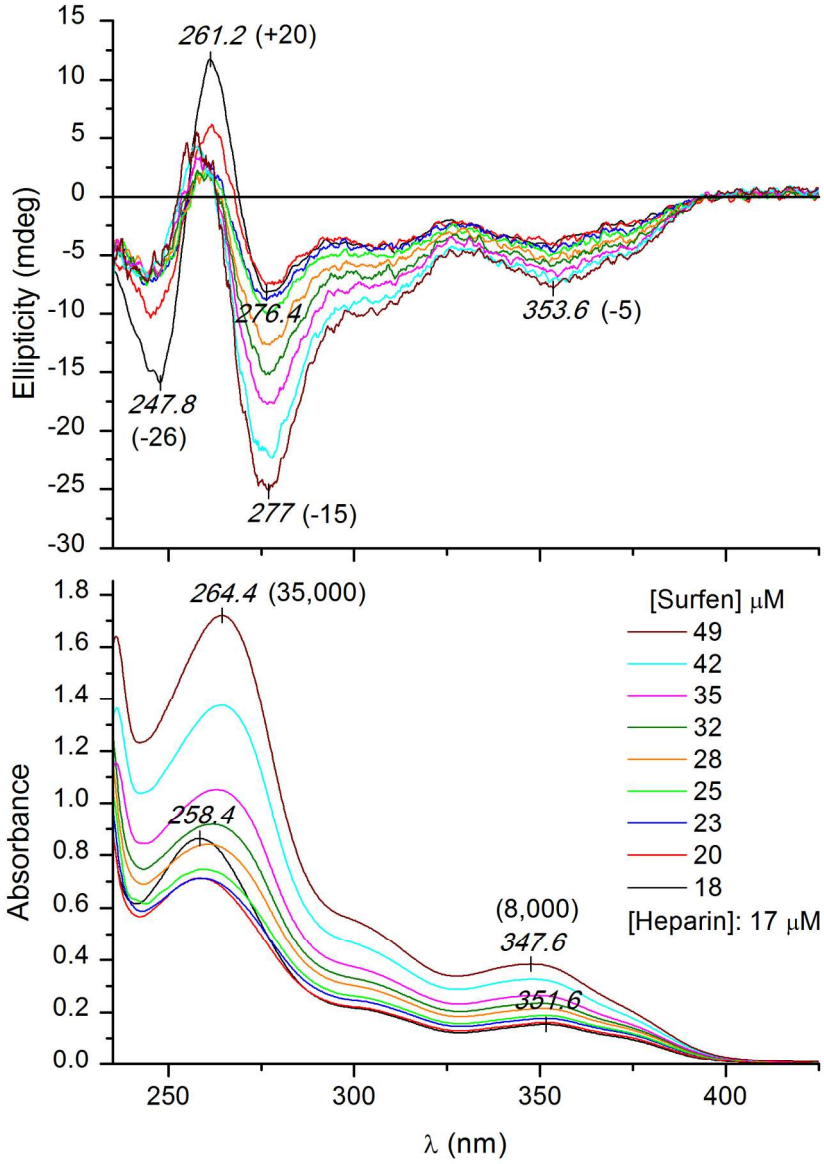

Figure 4
CD and UV absorption spectra of surfen measured at drug/disaccharide binding ratios above of 1. Molar dichroic absorption (±Δε) and molar absorption (ε) coefficients of peak extrema calculated by using actual surfen concentration of the sample are shown in parentheses.
597x838mm (72 x 72 DPI)

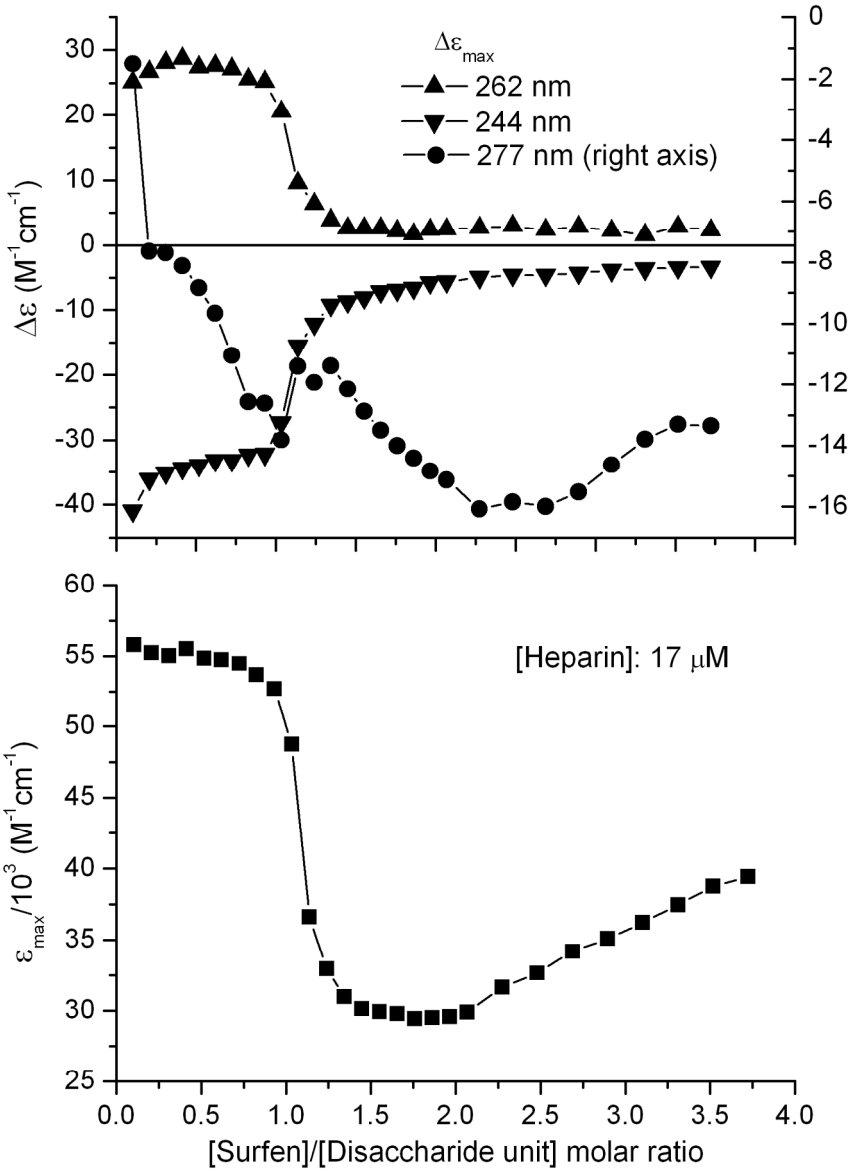

Figure 5
Changes of $\Delta\varepsilon_{max}$ and ε_{max} (259-265 nm) values measured during the titration of 17 μM heparin solution with surfen.
612x848mm (72 x 72 DPI)

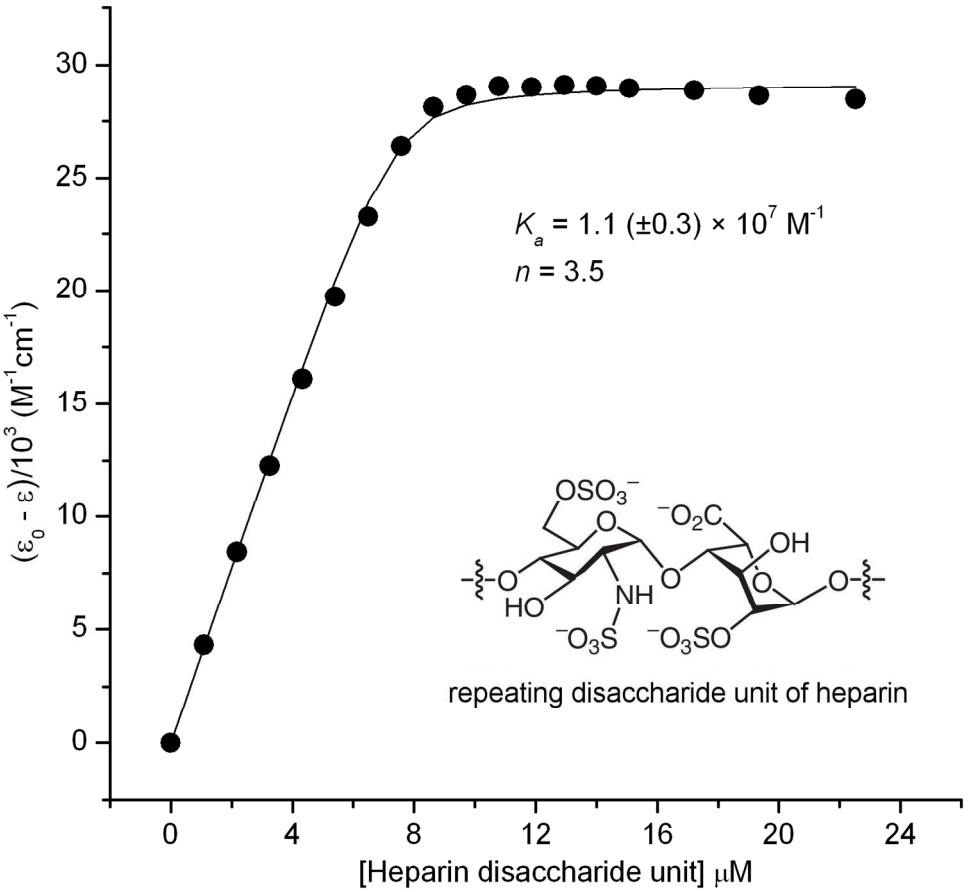

Figure 6
UV absorption changes plotted against the heparin concentration of the sample solution containing 26 μM surfen (20 mM Tris-HCl, pH 7.4, 50 mM NaCl, 25 °C). ε_0: molar absorption coefficient of the main UV peak of surfen measured in heparin-free buffer solution; ε: molar absorption coefficient of surfen measured in the presence of heparin. Solid line is the results of non-linear curve fitting analysis. Estimated association constant (K_a) and the number of surfen binding sites (n) per a disaccharide unit are shown.
615x570mm (72 x 72 DPI)

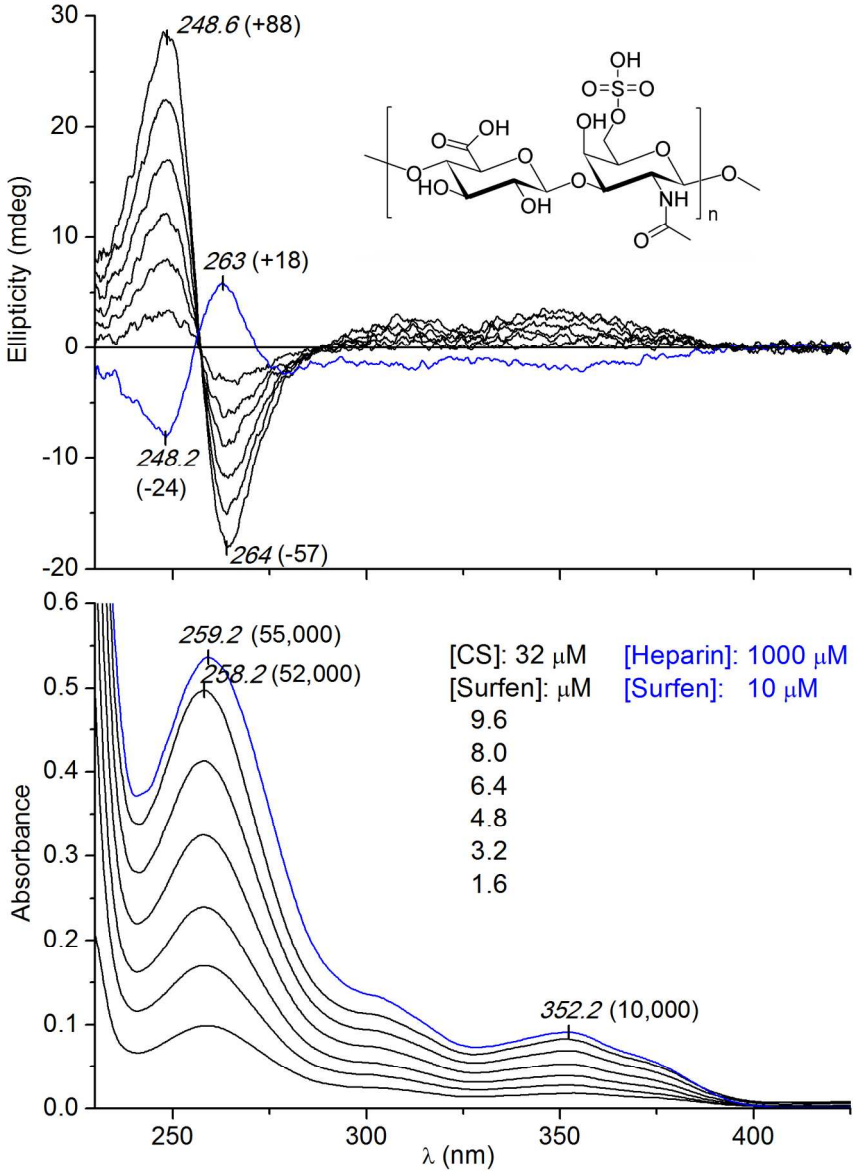

Figure 7
Comparison of the CD and absorption spectra of surfen measured with heparin (blue) and chondroitin-6-sulfate (black). Molar dichroic absorption (±Δε) and molar absorption (ε) coefficients of peak extrema calculated by using actual surfen concentration of the sample are shown in parentheses. Inset displays the repeating disaccharide unit of CS composed of β-D-glucuronic acid and *N*-acetyl-β-D-galactosamine 6-*O*-sulfate.
600x811mm (72 x 72 DPI)

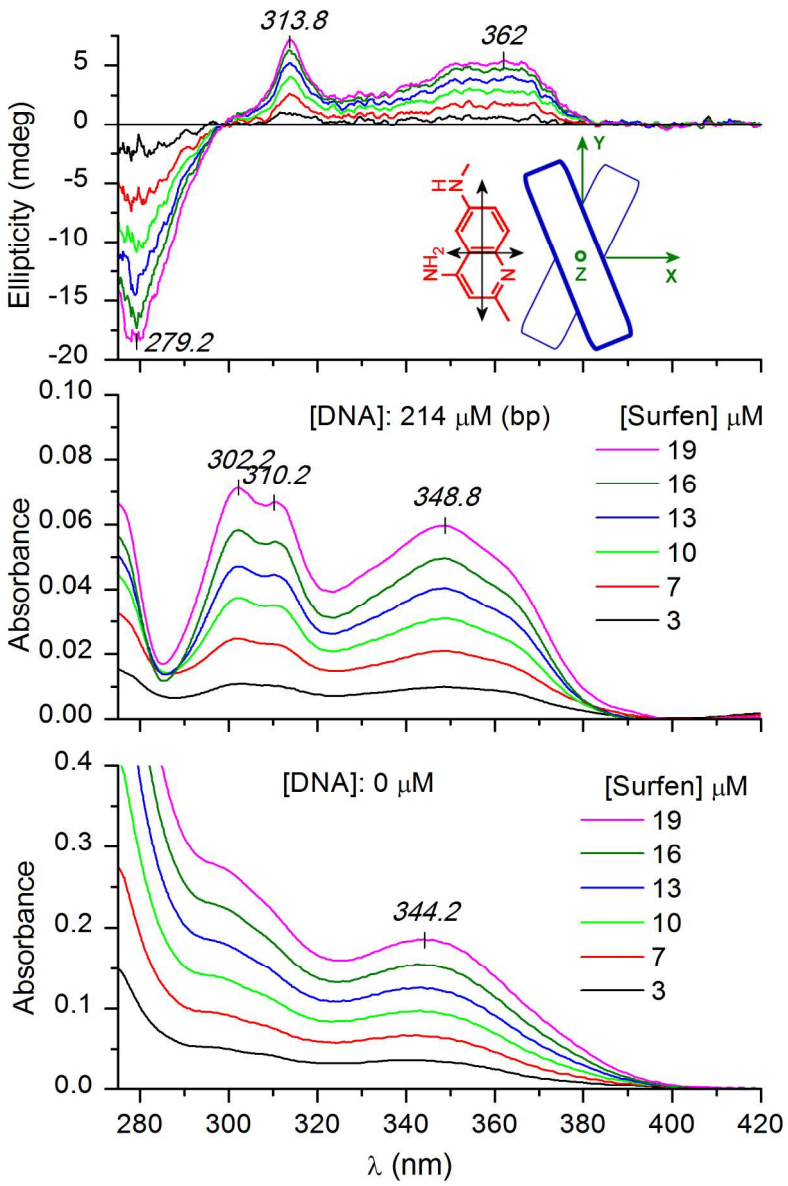

Figure 8

CD and UV absorption spectra of surfen measured at high molar excess of calf-thymus DNA (spectra were corrected by the CD and UV contribution of blank DNA solution). Bottom panel: UV curves of surfen obtained in DNA-free buffer solution at the same concentrations. Inset: schematic diagram of the proposed intercalation geometry of the aminoquinoline ring of surfen. Blue rectangles represent two successive base pairs of right-handed B-DNA. **X** and **Y** are the short and long axis of the intercalation pocket. The **Z** axis is perpendicular to the plane of the paper and is identical with the DNA helix axis (green circle). Double-headed arrows indicate the approximate polarization direction of the shorter-wavelength (λ < 300 nm, long axis) and longer wavelength (λ > 300 nm, short axis) π-π* transitions of the aminoquinoline moiety.
597x869mm (72 x 72 DPI)

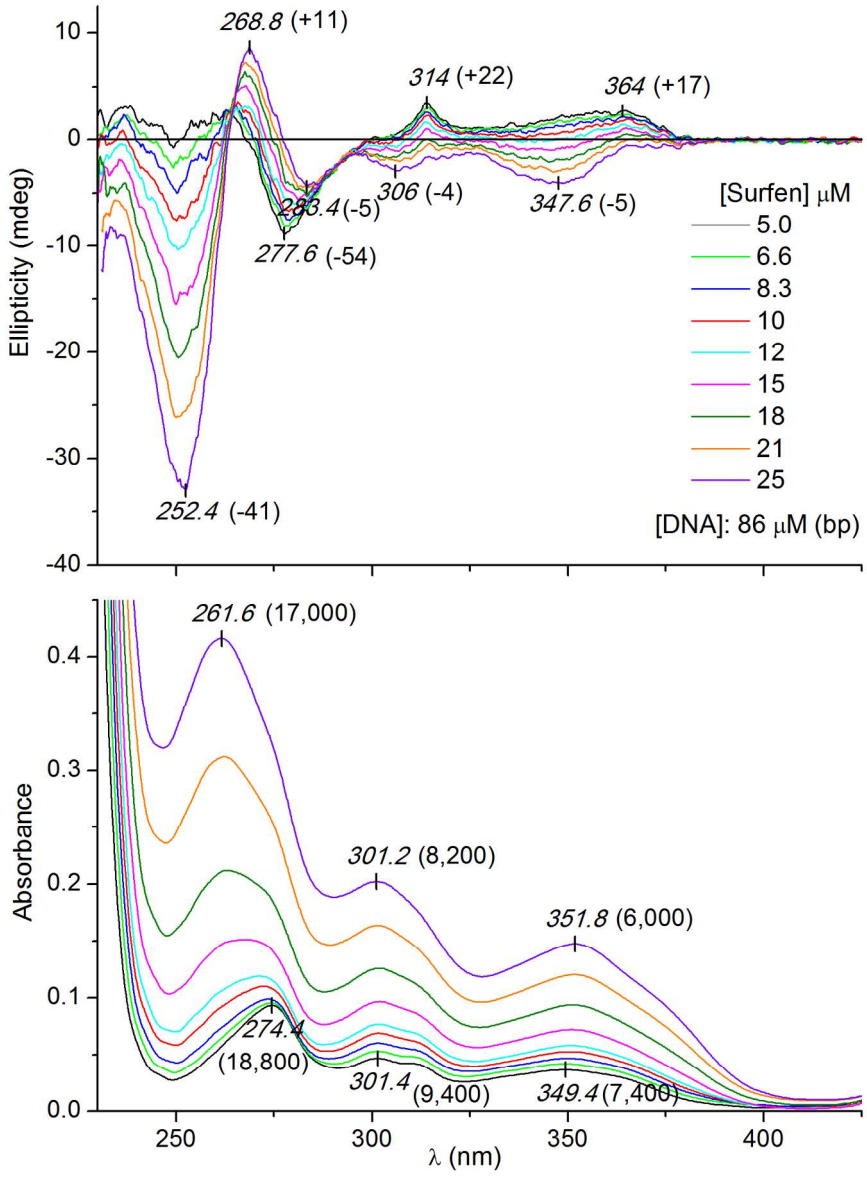

Figure 9
CD and UV absorption spectra of surfen measured at moderate excess of calf-tymus DNA (spectra were corrected by the CD and UV contribution of blank DNA solution). Molar dichroic absorption (±Δε) and molar absorption (ε) coefficients of peak extrema are shown in parentheses.
627x846mm (72 x 72 DPI)